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Observations on tissue schizogony and sporogony of rodent malaria

BY

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Summary — The study of the exo-erythrocytic schizogony of strains of *P. berghei* showed that the maturation time and size of the schizogonic forms are the same for the proposed *P. b. berghei* and *P. b. yoelii* and therefore are not reliable criteria for differentiation of the subspecies of *P. berghei*.

Furthermore, the size of the sporogonic forms seemed to be of doubtful significance as a criterion for differentiation.

Seventeen years after the discovery of *P. berghei* by Vincke and Lips (1948) in Congo, Yoeli, thanks to incessant efforts and perseverance, obtained regular cyclical transmission and observed the tissue schizonts of rodent malaria for the first time in 1965.

A new strain of *P. vinckei* Rodhain 1952 recently allowed Bafort (1967, 1968) to find tissue schizonts and to compare the results of sporogony and schizogony of rodent plasmodia as described by different investigators.

In the meantime *P. chabaudi* Landau 1965 was described and new strains of *berghei* and *vinckei* groups were isolated in other African countries (Bruce-Chwatt and Gibson, 1955; Landau and Chabaud, 1965; Adam and Landau, Chabaud, 1966; Killick-Kendrick *et al.*, 1968). The pre-erythrocytic forms of *P. chabaudi* and *P. b. yoelii* were already described (Landau and Killick-Kendrick, 1966; Garnham, Landau and Killick-Kendrick, 1966). Bafort (1968), however, has relegated *P. chabaudi* to a sub-species of *P. vinckei, P. v. chabaudi* (Landau, 1965, nov. Taxon).

An attempt of differentiation into species and subspecies was published by Garnham and his collaborators (1966). It was based on vertebrate host, invertebrate host, blood forms, exo-erythrocytic forms, sporogony, optimal temperature, serology and susceptibility of rats and hamsters.

Vincke and Peeters (1953) found that sub-inoculations of blood from rats injected with *P. berghei* sporozoite-infected salivary glands were positive after the second day. Yoeli and collaborators

(1965) demonstrated mature exo-erythrocytic schizonts of *P. berghei*, Strain NK 65, in the liver of a baby hamster after 51 hours. Later, Garnham and collaborators (1966) found a maturation time of 50.30 hours for the same strain. The NK 65 strain of *P. b. berghei* has been isolated from *A. dureni*, caught in the forest gallery of the river Kisanga near Elisabethville and sent by plane to New York in 1965.

All original strains of *P. berghei* have lost their ability to produce viable gametocytes and no tissue schizogony has been recorded, except for a pyrimethamine-resistant substrain of the strain SP 11, isolated in 1961, and which develops chronic infection in mice, resulting in a very high survival rate (Vincke *et al.*, 1965; Scheepers-Biva *et al.*, 1967). Gametocytes were regularly observed in blood smears and attempts were made to infect mosquitoes. Good salivary gland infections of *A. stephensi* were obtained with a strain maintained only through blood passages for several years. Mice infected by sporozoites received high doses of pyrimethamine without any decraese in parasitaemia (Bafort *et al.*, in press).

During the recent years three new strains of *P. berghei* have been isolated in Katanga. Two of them originate from *A. dureni*; one caught in the forest gallery of the river Kasapa in Elisabethville (strain ANKA), the second in Kamena, a locality where Vincke isolated *P. vinckei* in 1952. The third strain was obtained by blood sub-inoculation from a wild-caught *Thamnomys* also in Kamena. By accident we lost the last two strains before completing the study of tissue schizogony.

A fact of considerable interest is that the strains ANKA and NK 65 were isolated in the same way in Elisabethville during the same rain-season 1965. In fact one of the authors (J. B.) sent various samples of hundreds of *A. dureni*, caught in the forest galleries Kisanga and Kasapa near Lubumbashi (previously Elisabethville) to Yoeli in New York, to Davidson in London and to Vincke in Antwerp during the months January, February and March 1965.

The study of the sporogony of *P. b. berghei* strain ANKA has already been described. (Vincke *et al.*, 1966; Vincke and Bafort, 1968). Today this strain has been maintained exclusively by 48 cycles of consecutive mosquito-transmissions. Some results of the tissue schizogony are given in the present paper.

Material and methods

As vertebrate host laboratory-bred *Thamnomys surdaster*, hamsters, white mice (strains NMRI, Gif TB, Krefeld) and parasite-free white rats were used; as invertebrate host *A. stephensi*. After the infective blood meal the mosquitoes were incubated at a temperature of 21 °C and a relative humidity of 82 %.

Sporozoites of strain ANKA were inoculated into a tail vein of the animals except for the hamsters which were exposed to bites of numerous infected mosquitoes for a short period. The sporozoite suspension was prepared by a method described by Vincke and Bafort (1968) or by mass dissection, Biopsies and autopsies of the livers, taken at ½ hour's intervals, were fixed in Carnoy's fluid for three hours and stained by the Giemsa-colophonium method as described by Bray and Garnham (1962). The strains of plasmodium used were the Anka strain of *P. b. berghei,* the 17 X strain of *P. b. berghei* and the 67 strain of *P. v. vinckei.*

Results

Sub-inoculation of blood from white rat 163, 43 hours after intravenous inoculation of 400,000 sporozoites from infected mosquitoes (batch 1148-1149) showed that the first pre-erythrocytic schizonts of *P. b berghei* are mature at 43 hours. Mice which were inoculated with blood samples from mouse 77, infected with 216,000 sporozoites (batch 1127) of the same strain, developed a parasitaemia after inoculation of blood taken after 45 hours. In hamster 72 the shortest maturation time averaged 47 hours (46.15 h - 47.45 h) after inoculation of sporozoites.

Landau and Killick-Kendrick (1966) described the prepatent period of sporozoite-induced infections of *P. b. yoelii* as 43 hours. However sub-inoculations of blood from *Thamnomys* 1350 after a single intravenous inoculation of 1,500,000 sporozoites became positive at 42.30 hours.

The exo-erythrocytic forms of *P. v. vinckei* strain 67 matured in the liver of *Thamnomys* 2583 in 53 hours. Sub-inoculations of blood in mice became positive after 53 hours although mature ruptured schizonts were present after 52 hours (Bafort, 1968). It seems as though the period of maturation should be longer in white mice. Sub-inoculations of blood from mice infected with sporozoites gave rise to infection in mice only after 61 hours in spite of the presence of mature schizonts as early as 54 hours and of parasitized red blood cells in the liver at 56 hours.

The prepatent period of sporozoite induced infections of rodent malaria also shows variations from one animal to another if only one species of vertebrate host is concerned, as we found for rat 73 and rat 76 infected with *P. b. berghei* respectively 45 and 46 hours after inoculation. Sub-inoculations of blood from *Thamnomys* 2569 and 2540, both infected with *P. v. vinckei,* gave 53.30 hours and 54 hours respectively.

In the same way the measurements of tissue schizonts of *P. b. berghei* strain ANKA differ from those of the strain NK 65 as given by Yoeli and Most (1965), Garnham *et al.* (1966), Landau *et al.* (1966) and Yoeli *et al.* (1966). We found a mean diameter of 25 μ (19-34 μ) in the liver of *Thamnomys* 162 at 43 hours,

of 29 μ (20-42 μ) in rat 76 at 48 hours and of 25 μ (17-34 μ) in mouse 46 at 43 hours. Yoeli and collaborators (1966) gave 47 μ in tree rat, 26 μ in rat and 25 μ in mice at 51 hours. Garnham *et al.* (1966) described a mean diameter of 26 μ in rat at 50 hours.

Yoeli and his collaborators (1966) found a distinct polymorphism and a difference in size among the pre-erythrocytic forms of *P. b. berghei* in different hosts. The largest forms of tissue schizogony of *P. v. vinckei* strain 67 were also described in tree rat : a mean diameter of 37 μ at 53 hours and 45 μ at 58 hours in *Thamnomys* against 26 μ in mice after 54 hours (Bafort, in press).

The oocysts of *P. b. berghei* strain ANKA also differ in size from those of the strain NK 65 given by Garnham and collaborators (1966) : 34 to 40 μ at 21 °C. For the ANKA strain mature oocysts range from 34 μ up to 55 μ at 21 °C and healthy sporogony is possible at 23 °C.

Discussion

Two strains of *P. b. berghei,* ANKA and NK 65, present mature exo-erythrocytic schizonts after respectively 43 and 50.30-51 hours. Both strains were isolated in Elisabethville in 1965 and the former shows more similarity to *P. b. yoelii* from R.C.A. (prepatent period 43 hours). This difference in maturation time of the tissue schizogony was one of the criterions used to distinguish the strains of *P. berghei* from Katanga from those from R.C.A. Moreover the size of the mature oocysts of strain ANKA seems to be intermediate between those of NK 65 and *P. b. yoelii.* Furthermore Yoeli and collaborators (1966) reported liver schizonts of the strain NK 65 larger than those of *P. b. yoelii* (47μ against 37 μ).

If these factors should be taken into account to differentiate plasmodia in sub-species it must be emphasized that many similarities can be found between *P. b. berghei* and *P. b. yoelii.*

As for the number of merozoites in exo-erythrocytic schizonts of *P. b. berghei* Garnham (1966) stated 4,000 whereas Yoeli and collaborators (1966) estimated as high as 10,000 to 18,000.

The study of the sporogony of *P. b. yoelii* by Wéry (1966) and of *P. v. vinckei* strain 67 by Bafort (in press) shows a possible influence of temperature, vertebrate host and invertebrate host on the size of sporogonic forms. The measurements of the sporozoites of *P. v. vinckei* strain 67 ranged from 11 μ up to 21μ. Fully mature oocysts may be found in a range from 38 to 70 μ.

In other respects it is worth mentioning that Vincke and Peeters already in 1952 noticed that a strain of *P. berghei,* SP 137, from Elisabethville did not present the same characteristics as the others :

« *Elle semblait cependant, tout comme le* P. vinckei, *faiblement pathogène pour le rat blanc.* » (Vincke and Peeters 1953, p. 88). This observation suggests a less virulent strain exactly as the one isolated by Landau in R.C.A and named *P. b. yoelii*. So it seems as though in Katanga one does not only find strains with exo-erythrocytic schizonts maturing in the same time as *P. b. yoelii*, but also strains which are less virulent like this subspecies.

Although it is not intended to correct the conception of present classification of rodent malaria, the maturation time and size of schizogony and sporogony forms seem to be subjected to various factors (e.g. vertebrate host, invertebrate host and temperature) making difficult to estimate their real taxonomic value.

To this purpose we propose only to take into consideration studies made on the natural host under strictly standardized circumstances.

Résumé — **Observations sur la schizogonie tissulaire et la sporogonie du paludisme des rongeurs.**

L'étude de la schizogonie exo-érythrocytaire de souches de *P. berghei* a montré que le temps de maturation et la dimension des formes exo-érythrocytaires sont les mêmes pour *P. b. berghei* et *P. b. yoelii* et ne possèdent donc aucune valeur comme critères de différentiation.

D'autres caractères tels que la dimension des formes sporogoniques et autres semblent également ne posséder qu'une signification douteuse comme critères de différentiation.

Samenvatting — **Observaties over weefsel schizogonie en sporogonie bij knaagdieren malaria.**

Bij de studie van de exo-erythrocytaire schizogonie van stammen van *P. berghei* bleek dat de rijpingstijden en de afmeting der exo-erythrocytaire vormen gelijk zijn voor *P. b. berghei* en *P. b. yoelii*. Deze eigenschappen zijn dus als differentiatiekenmerk waardeloos.

Verder bleken andere eigenschappen als de afmeting der sporogonieën e.d. eveneens slechts van twijfelachtige betekenis als differentiatiecriteria.

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BIBLIOGRAPHY

Adam, J. P., Landau, I. and Chabaud, A. G., Découverte dans la région de Brazzaville de rongeurs infectés par des plasmodiums. C. R. Acad. Sc. Paris, 1966, **263**, 140.

Bafort, J., La transmission cyclique du *Plasmodium vinckei*. Ann. Soc. belge Méd. trop., 1967, **47**, 3, 271.

Bafort, J., Primary exo-erythrocytic forms of *Plasmodium vinckei*. Nature, 1968, **217**, 5135, 1264.

Bafort, J., Etude du cycle de *Plasmodium v. vinckei*. Ann. Soc. belge Méd. trop., in press.

Bafort, J. and Timperman, G. Etude comparative d'une lignée de souris résistantes au *P. berghei*. Z. Tropenmed. Parasit, in press.

Bray, R. S. and Garnham, P. C. C., The giemsa-colophonium method for staining protozoa in tissue sections. Indian. J. Malar., 1962, **16**, 153.

Bruce-Chwatt, L. J. and Gibson, F. D., A plasmodium from a nigerian rodent. Trans. R. Soc. trop. Med. Hyg., 1955, **49**, 9.

Garnham, P. C. C., Landau, I. and Killick-Kendrick, R., Primary exo-erythrocytic schizonts of three rodent malaria parasites. Trans. R. Soc. trop. Med. Hyg., 1966, **60**, 1, 4-5.

Garnham, P. C. C., Landau, I., Killick-Kendrick, R. and Adam, J. P., Répartition et caractères différentiels des Plasmodiums de Muridés. Bull. Soc. Path. Exotique, 1967, **60**, 118.

Killick-Kendrick, R., Schute, G. T., Lambo, A. and Guy, M. W., Rodents Plasmodia of Nigeria. Trans. R. Soc. trop. Med. Hyg., 1968, **62**, 2.

Landau, I, and Chabaud, A. G., Infection naturelle par deux plasmodiums du rongeur *Thamnomys rutilans* en République Centre Africaine. C. R. Acad. Sc. Paris, 1965, **260**, 230.

Landau, I. and Killick-Kendrick, R., Rodent Plasmodia of the Republique Centrafricaine, The sporogony and tissue stages of *Plasmodium chabaudi* and *P. berghei yoelii*. Trans. R. Soc. trop. Med. Hyg., 1966, **60**, 5, 633.

Rodhain, J., *Plasmodium vinckei* n. sp. Un deuxième plasmodium parasite de rongeurs sauvages au Katanga. Ann. Soc. belge Méd. trop., 1952, **32**, 275.

Scheepers-Biva, M., Bafort, J. and Vincke, I. H., Untersuchungen zur resistenzbildung gegen *Plasmodium berghei* bei Maüsen. 1967, **18**, 1, 45-47.

Vincke, I. H. and Lips, M., Un nouveau plasmodium d'un rongeur sauvage du Congo : *P. berghei*. Ann. Soc. belge Méd. trop., 1948, **28**, 97-102.

Vincke, I. H. and Peeters, E., Observations sur la transmission de sporozoites d'anophèles dureni à des rongeurs sauvages et de laboratoire. Ann. Soc. belge Méd. trop., 1953, **33**, 87-93.

Vincke, I. H., Le paludisme des rongeurs en Afrique. Ann. Soc. belge Méd. trop., 1964, **44**, 3, 579-586.

Vincke, I. H. and Scheepers-Biva, M., Survie et guérison spontanée chez les souris infectées de *Plasmodium berghei*. Ann. Soc. belge Méd. trop., 1965, **45**, 313-324.

Vincke, I. H., Bafort, J. and Scheepers-Biva, M., Observations récentes sur la transmission cyclique du *Plasmodium berghei*. Ann. Soc. belge. Méd. trop., 1966, **46**, 3, 327.

Vincke, I. H. and Bafort, J., Méthodes de standardisation de l'inoculum de sporozoites de *Plasmodium berghei*. Ann. Soc. belge Méd. trop., 1968, **48**, 2, 181-194.

Vincke, I. H. and Bafort, J., Résultats de deux ans d'observations sur la transmission cyclique de *Plasmodium berghei*. Ann. Soc. belge Méd. trop., 1968, sous presse.

Wéry, M., Etude du cycle de *Plasmodium berghei yoelii* en vue de la production massive de sporozoites viables et de formes exo-érythrocytaires. Ann. Soc. belge Méd. trop., 1966, **46**, 6, 755-788.

Yoeli, M. and Most, H., Pre-erythrocytic development of *P. berghei*. Nature, 1965, **205**, 7105.

Yoeli, M., Upmanis, R. S., Vanderberg, J. and Most, H., Life cycle and patterns of development of *P. berghei* in normal and experimental hosts. Mil. Med., 1966, **131**, 900.